Urinary Tract Microbiota Profiling Experiments

APPLICATION GUIDE

TaqMan™ Assays for urinary tract microbiota profiling experiments in TaqMan™ OpenArray™ Plate-format

for use with:
TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate
Custom TaqMan™ OpenArray™ Plates
QuantStudio™ 12K Flex instrument with OpenArray™ block (AccuFill™ System)

Catalog Numbers A39900
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Revision C.0

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0017750

Revision	Date	Description
C.0	29 March 2021	Updated information for TaqMan™ Universal DNA Spike In Control.
B.0	9 August 2018	• Added information for the TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate. • Added references for additional user documentation. • Updated DNA isolation protocol per V&V testing.
A.0	25 March 2018	New document.

Contents

1 Introduction and workflow overview

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

This guide describes the OpenArray™ plate high-throughput, sample-to-result workflow for urinary tract microbiota profiling. The workflow uses:

- MagMAX™ DNA Multi-Sample Ultra Kit for DNA isolation from urine samples
- OpenArray™ plates with TaqMan™ Assays for urinary tract microbiota profiling
- QuantStudio™ 12K Flex instrument with OpenArray™ block (AccuFill™ System)

Urinary tract microbiota profiling

Microorganism-specific TaqMan™ Assays offer a rapid and accurate approach to investigate and monitor urinary tract microbiome composition and dynamics.

We offer a collection of qualified TaqMan™ Assays that are optimized for detection of urinary tract microbes (see "TaqMan™ Assays for urinary tract microbiota profiling" on page 8). The TaqMan™ Assay design and their target sequences have undergone rigorous bioinformatics selection and analysis to ensure maximum strain coverage and minimal off-target cross-reactivity. Qualified TaqMan™ Assays for urinary tract microbiota profiling demonstrate accurate, reproducible performance in multiple rounds of testing for sensitivity and specificity. The assays perform well with DNA isolated from urine samples using optimized MagMAX™ DNA Multi-Sample Ultra Kit protocols.

Additional TaqMan™ Assays for microbial targets are available from our predesigned assay collection. For Custom TaqMan™ Assays contact **QuantStudioFrontDesk@thermofisher.com**.

Workflow: TaqMan™ urinary tract microbiota profiling experiments

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2 Background and tools for assay selection

TaqMan™ Assays

TaqMan™ Assays for urinary tract microbiota profiling consist of a pair of unlabeled PCR primers and a TaqMan™ probe with a FAM™ dye label on the 5' end and minor groove binder (MGB) and nonfluorescent quencher (NFQ) on the 3' end.

For more information about real-time PCR and TaqMan™ Assays, visit **thermofisher.com/qpcreducation**.

TaqMan™ Assays for urinary tract microbiota profiling

All of the TaqMan™ Assays for urinary tract microbiota profiling, including the controls, are included in the TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate. The assays can also be configured with Custom TaqMan™ OpenArray™ Plate (see "TaqMan™ OpenArray™ Plate products and formats" on page 9). For optional reference and controls, see "Optional controls" on page 9

For more information about available TaqMan™ Assays for urinary tract microbiota profiling, visit **thermofisher.com/utm**.

Table 1 TaqMan™ Assays for urinary tract microbiota profiling

Assay ID	Classification	Target organism name
Ba04932084_s1	Bacteria	*Acinetobacter baumannii*
Ba04932088_s1	Bacteria	*Citrobacter freundii*
Ba04932080_s1	Bacteria	*Enterobacter aerogenes*
Ba04932087_s1	Bacteria	*Enterobacter cloacae*
Ba04646247_s1	Bacteria	*Enterococcus faecalis*
Ba04932086_s1	Bacteria	*Enterococcus faecium*
Ba04646242_s1	Bacteria	*Escherichia coli*
Ba04932079_s1	Bacteria	*Klebsiella oxytoca*
Ba04932083_s1	Bacteria	*Klebsiella pneumoniae*
Ba04932078_s1	Bacteria	*Morganella morganii*
Ba04932076_s1	Bacteria	*Proteus mirabilis*
Ba04932082_s1	Bacteria	*Proteus vulgaris*
Ba04932077_s1	Bacteria	*Providencia stuartii*
Ba04932081_s1	Bacteria	*Pseudomonas aeruginosa*
Ba04932085_s1	Bacteria	*Staphylococcus saprophyticus*
Ba04646276_s1	Bacteria	*Streptococcus agalactiae*
Fn04646233_s1	Yeast	*Candida albicans*
***(Optional)* Control assays**[1]		
Hs04930436_g1	Control	Human RNase P RPPH1 gene[2]
Ac00010014_a1	Control	Xeno™[3]

[1] Included in the TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate.
[2] Use to assess sample adequacy.
[3] Use to control for nucleic acid recovery in sample preparation process.

Optional controls

(Optional) TaqMan™ Universal DNA Spike In Control

The TaqMan™ Universal DNA Spike In Control (Cat. No. A39175) is an exogenous Xeno™ DNA process control that can be used to monitor the recovery efficiency for the DNA extraction and purification process. The control also indicates the presence of PCR inhibitors in molecular detection workflows. This control is of particular importance when working with urine samples that can have a higher frequency of inhibition.

This product contains a sequence for AmpC beta-lactamase and may amplify for any assays designed to detect this antibiotic resistance target. For example, TaqMan™ Assay ID Ba04646128_s1 will amplify in any sample that contains the TaqMan™ Universal DNA Spike In Control.

TaqMan™ Universal DNA Spike In Control is supplied at a concentration of 200,000 copies/µL. The control is added during DNA isolation and is then carried through the remainder of the urinary tract microbiota profiling workflow. Coupled with the proprietary TaqMan™ Assay for the Xeno™ DNA control (Assay ID Ac00010014_a1), this verification layer helps ensure that PCR results are accurate, and it reduces the likelihood of false negatives.

For information about TaqMan™ Universal DNA Spike In Control, see *TaqMan™ Universal DNA Spike In Control Product Information Sheet* (Pub. No. MAN0017852).

(Optional) Amplification control

The TaqMan™ Urinary Tract Microbiota Amplification Control (Cat. No. A39174) contains a linearized multi-target plasmid with target sequences for each available urinary tract microbiota profiling assay. The plasmid also contains target sequences for Xeno DNA and human RNase P RPPH1 genes, for a general control for the sample preparation process. The TaqMan™ Urinary Tract Microbiota Amplification Control can be included in urinary tract microbiota profiling experiments to verify assay performance and to help with troubleshooting.

For information about the amplification control, see *TaqMan™ Urinary Tract Microbiota Amplification Control Product Information Sheet* (Pub. No. MAN0017753).

TaqMan™ OpenArray™ Plate products and formats

TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate

The TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate (Cat. No. A39900) contains pre-plated, dried down TaqMan™ Assays for urinary tract microbiota profiling. For the complete lists of assays included with the TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate, see Table 1.

Contents and storage

Table 2 TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate (Cat. No. A39900)

Component	Amount	Storage
TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate	1 plate	–25°C to –15°C

Custom TaqMan™ OpenArray™ Plate products and formats

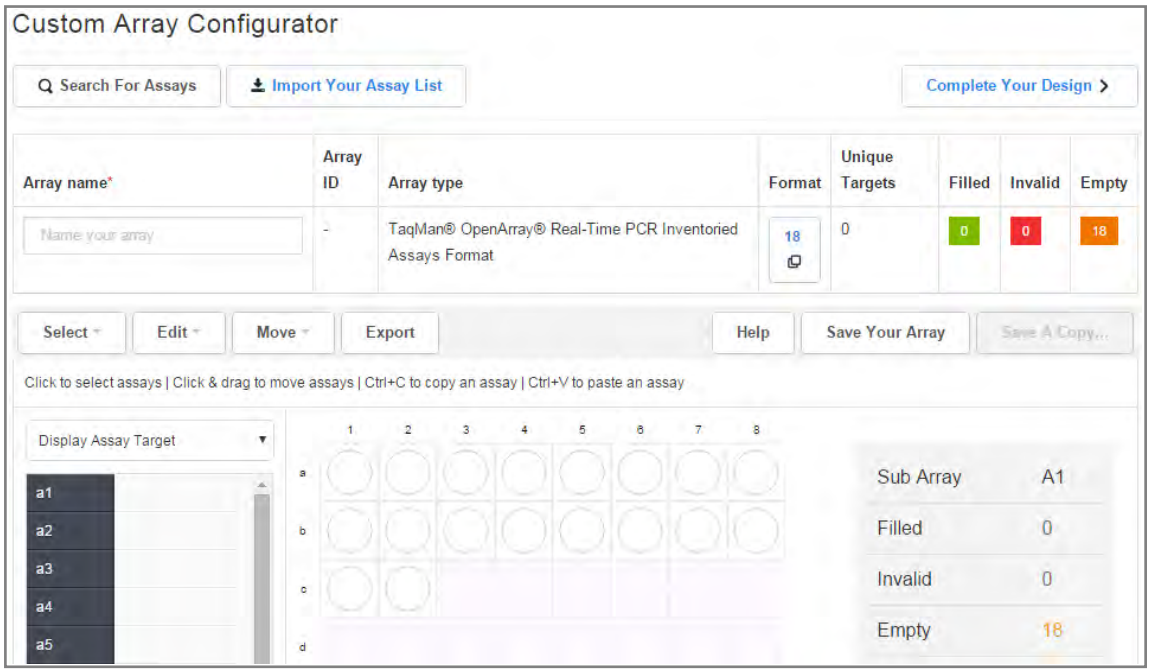
Custom TaqMan™ OpenArray™ Plates contain pre-plated, dried down TaqMan™ Assays for urinary tract microbiota profiling.

Array format	Number of assays	Maximum number of samples
18	18	48
56	56	48
112	112	24

Note: We recommend at least three technical replicates of each reaction.

Configure and order custom TaqMan™ OpenArray™ plates

1. Go to **thermofisher.com/order/custom-array**.
2. For array type, select **TaqMan™ OpenArray™ Real-Time PCR Inventoried Assays Format**.
3. In the table, click **Select** to configure a plate with the desired array format.
   The **Custom Array Configurator** screen displays.



4. Enter the custom array name in the **Array name** text field.
5. Click **Import Your Assay List**, then upload or copy-paste the assay information:
   - Under **Upload a list of Assay IDs**, click **Choose File**, then select a tab-delimited text file (TXT) containing Assay IDs.
     or
   - Under **Enter a list of Assay IDs**, paste the Assay IDs, then click **Import Entered List**.
6. Follow the on-screen instructions to configure the assays on the plate.
7. *(Optional)* Click **Save Your Array** at any time to save the array configuration to your Thermo Fisher Scientific account.
8. When the plate is configured, click **Complete Your Design**, then follow the on-screen instructions to complete the order.

3 Isolate DNA from urine research samples using the MagMAX™ DNA Multi-Sample Ultra Kit

Compatible sample collection and storage

Collect urine samples using BD™ Vacutainer® urine collection cups and tubes.

- Compatible urine samples:
  - Unstabilized urine that is collected in sterile containers (BD™ Cat. No. 364975)
  - Urine that is collected and stored in Urine Analysis (UA) tubes (BD™ Cat. No. 364992)
  - Urine that is collected and stored in Culture and Sensitivity (C&S) tubes (BD™ Cat. No. 364951)
- *(Optional)* Store samples according to the instructions provided with the collection container, or use the following storage conditions:
  - Store at 4°C for up to one week.
  - Store at –80°C for long-term storage. We recommend storing samples in smaller volumes to prevent multiple freeze/thaw cycles.

Contents and storage

Table 3 MagMAX™ DNA Multi-Sample Ultra Kit

Contents	Cat. No. A25597 (500 rxns)	Cat. No. A25598 (2,500 rxns)	Storage
Proteinase K	4 mL	5 × 4 mL	–25°C to –15°C
PK Buffer	96 mL	5 × 96 mL	15°C to 30°C
Multi-Sample DNA Lysis Buffer	100 mL	5 × 100 mL	
DNA Binding Beads	8 mL	5 × 8 mL	2°C to 8°C
RNase A	2 × 1.25 mL	10 × 1.25 mL	–25°C to –15°C
Nuclease-free Water	100 mL	5 × 100 mL	15°C to 30°C
Wash Solution 1 Concentrate[1]	80 mL	5 × 80 mL	
Wash Solution 2 Concentrate[1]	162 mL	5 × 162 mL	
DNA Elution Buffer 1	25 mL	5 × 25 mL	
DNA Elution Buffer 2	25 mL	5 × 25 mL	

[1] Before use of the kit, prepare all applicable wash solutions as described on their bottles and in this protocol.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Table 4 Required materials and equipment not included with the kit

Item	Source
KingFisher™ Flex Magnetic Particle Processor	5400630
Equipment	
Plate shaker, capable of shaking plates at a minimum of 900 rpm	88880023
Analog Vortex Mixer	Fisher Scientific 02-215-365
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
(Optional) Magnetic Stand-96	AM10027

Item	Source
Plates and combs	
Deep Well Plates, one of the following:	
KingFisher™ Flex Microtiter Deep-Well 96 plates, sterile	95040460
MagMAX™ Express-96 Deep Well Plates	4388476
Standard Well Plates, one of the following:	
KingFisher™ 96 KF microplates	97002540
MagMAX™ Express-96 Standard Plates	4388475
Tip Combs, one of the following:	
KingFisher™ 96 tip comb for DW magnets	97002534
MagMAX™ Express-96 Deep Well Tip Combs	4388487
Other consumables	
MicroAmp™ Clear Adhesive Film	4306311
RNase-free Microfuge Tubes (2.0 mL)	AM12425
Conical tubes (15 mL)	AM12500
Conical tubes (50 mL)	AM12502
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
(Optional) Paraffin film	MLS
Reagents	
Ethanol, 200 proof (absolute)	MLS
Isopropanol, 100% (molecular grade or higher)	MLS

Table 5 Additional materials and equipment required for processing urine samples

Item	Source
Centrifuge, capable of spinning deep-well plates at 2,250 × *g*	Fisher Scientific 75-412-452
Laboratory incubator with slatted shelves, capable of maintaining 65°C	MLS
MagMAX™ Wash Solution 1 Concentrate	AM8504
B-PER™ Bacterial Protein Extraction Reagent	78243
Lysozyme Solution	90082

Table 5 Only for urinary tract (UTM)Additional materials and equipment required for processing urine samples *(continued)*

Item	Source
Zymolyase	Fisher Scientific 50-444-504
(Optional) TaqMan™ Universal DNA Spike In Control (Xeno™ DNA control)	A39175

Download the KingFisher™ Flex program (if needed)

The program required for this protocol is not pre-installed on the KingFisher™ Flex Magnetic Particle Processor.

1. On the MagMAX™ DNA Multi-Sample Ultra Kit web page, scroll down to the **Product Literature** section.
2. Click **A25597_UTM** to download the program to your computer.
3. See *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. No. N07669) and *BindIt™ Software User Manual* (Cat. No. N07974) for instructions for installing the program on the instrument.

Procedural guidelines

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Preheat an incubator to 65°C before each use of the kit.
- Use the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and 96-well standard heat block.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. The same MicroAmp™ Clear Adhesive Film can be used throughout the procedure, unless it becomes contaminated.
- If you use a plate shaker other than the recommended shaker, verify that:
  - The plate fits securely on your plate shaker.
  - The recommended speeds are compatible with your plate shaker. Ideal shaker speeds allow for thorough mixing without splashing.
- To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.
- Per-plate volumes for reagent mixes are sufficient for one plate plus overage. To calculate volumes for other sample numbers, refer to the per-well volume and add 5% overage.
- For convenience, you can extend the Proteinase K digestion to 30 minutes.

Before first use of the kit

- Prepare the Wash Solutions from the concentrates:
  - Add 25 mL of isopropanol to Wash Solution 1 Concentrate (from the kit). Mix the isopropanol and concentrate, then store at room temperature.
  - Add 132 mL of ethanol to Wash Solution 2 Concentrate (from the kit). Mix the ethanol and concentrate, then store at room temperature.
  - Add 70 mL of isopropanol to Wash Solution 1 Concentrate (separately purchased bottle, Cat. No. AM8504). Mix the isopropanol and concentrate, then store at room temperature.

- Reconstitute the zymolyase with 500 µL of the provided storage buffer (final concentration of 4 U/µL), vortex to mix, then store at –20°C.

  For more information, see the documentation provided with the zymolyase.

Set up the sample layout

The sample plate layout provides sample tracking from the 96-well plate used for DNA isolation to the 96-well sample plate CSV file used for import into the OpenArray™ Sample Tracker Software.

Set up the sample plate layout using the CSV file described in the following table.

Note: We recommend at least three technical replicates of each reaction.

Tool	Source	Description
`96-well Sample Plate 1.csv` template	On the computer on which the OpenArray™ Sample Tracker Software is installed: `C:\Program Files\Applied Biosytems\Sample Tracking Utility\examples`	Contains a sample layout tab.

Concentrate the samples

1. Gently invert, shake, or swirl the sample contents to ensure thorough mixing of the sample.
2. Following the sample layout, transfer 1 mL of sample to the wells of a deep-well plate.
3. Seal the plate with a clear adhesive film, then centrifuge the plate at 2,250 × *g* for 15 minutes to concentrate the samples.
4. **IMPORTANT!** There may not be an obvious pellet. If a pellet is visible, be careful not to disturb the pellet.

   After centrifugation, carefully remove, then discard the supernatant.

   a. Set a P1000 pipette (or similar) to 900 µL.

      Note: We recommend using a manual P1000 multichannel pipette. An electronic multichannel pipette can also be used at low speeds (<6 on Ranin pipettes).

   b. Angle the pipette so that the pipette tips sit at the bend from square to conical in the plate well.

   c. Carefully remove supernatant, then discard.

   d. Repeat substep 4b and substep 4c.

   e. Visually inspect the samples to ensure that all urine has been removed.
      If > 30 µL of urine remains, repeat substep 4b and substep 4c.

Digest the samples with the Preliminary Digestion Mix

1. Prepare sufficient Preliminary Digestion Mix according to the following table.

   IMPORTANT! Prepare the Preliminary Digestion Mix no more than 30 minutes before use and store on ice. Prolonged storage at room temperature can reduce its efficiency.

Component	Volume per well	Volume per plate
B-PER™ Bacterial Protein Extraction Reagent	185 µL	18.5 mL
Lysozyme Solution	10 µL	1 mL
Zymolyase solution (4 U/µL)	5 µL	0.5 mL
Total Preliminary Digestion Mix	**200 µL**	**20 mL**

2. Add 200 µL of Preliminary Digestion Mix to each sample well.
   (Optional) If large pellets are present, disperse by pipetting up and down 10–20 times.
3. Seal the plate with a clear adhesive film, then shake at 1,050 rpm for 3 minutes.

4. Incubate the plate for 15 minutes at 65°C.

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

During the incubation, prepare the PK Mix (next section).

Digest the samples with Proteinase K

1. Prepare sufficient PK Mix according to the following table, then invert several times to thoroughly mix components.

IMPORTANT! Prepare the PK Mix no more than 30 minutes before use and store at room temperature. Do not place PK Buffer or PK Mix on ice, to avoid precipitation.

Component	Volume per well	Volume per plate
Proteinase K	8 µL	0.8 mL
PK Buffer	42 µL	4.2 mL
Total PK Mix	**50 µL**	**5.0 mL**

2. When the incubation with Preliminary Digestion Mix is complete, add 50 µL of PK Mix to each sample well of the plate.

3. Seal the plate with a clear adhesive film, then shake the sealed plate at 1,050 rpm for 3 minutes.

4. Incubate for 15 minutes at 65°C.

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

Set up the processing plates

1. While the samples are incubating at 65°C, set up the Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	300 µL
Wash Plate 2	3	Deep Well	Wash Solution 1	300 µL
Wash Plate 3	4	Deep Well	Wash Solution 2	150 µL
Wash Plate 4	5	Deep Well	Wash Solution 2	150 µL
Elution Plate[2]	6	Standard	DNA Elution Buffer 1	30 µL
Tip Comb	7	Deep Well	Place a tip comb in the plate.	

[1] Position on the instrument

[2] The instrument prompts the user to add DNA Elution Buffer 2 to the Elution Plate, after incubation with DNA Elution Buffer 1.

2. *(Optional)* To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

Add Multi-Sample DNA Lysis Buffer, Bead/RNase A Mix, and isopropanol

1. *(Optional)* If condensation is present at the end of the 65°C incubation, briefly centrifuge the plate at 1,500 × *g* for 1–2 minutes.

2. Prepare sufficient Bead/RNase A Mix according to the following table.

IMPORTANT! Prepare the Bead/RNase A Mix no more than 1 hour before use and store on ice. Prolonged storage at room temperature can reduce its efficiency.

Vortex the DNA Binding Beads at moderate speed to form a uniform suspension before preparing the Bead/RNase A Mix.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
RNase A	5 µL	0.5 mL
Nuclease-free Water	19 µL	1.9 mL
Total Bead/RNase A Mix	**40 µL**	**4.0 mL**

3. Add 125 µL of Multi-Sample DNA Lysis Buffer to each sample.

4. *(Optional)* Add 10 µL of TaqMan™ Universal DNA Spike In Control (Xeno™ DNA control) to each sample.

   Note: For more information about the Xeno™ DNA control, see *TaqMan™ Universal DNA Spike In Control Product Information Sheet* (Pub. No. MAN0017852).

5. Vortex the Bead/RNase A Mix at moderate speed to ensure thorough mixing, then add 40 µL to each sample.

   If you see that the beads in the Bead/RNase A Mix are settling, vortex the mix again briefly before continuing to pipette.

6. Add 200 µL of isopropanol to each sample, then proceed immediately to process the samples on the instrument (next section).

Process samples on the instrument

1. Select the program on the instrument.
   - KingFisher™ Flex Magnetic Particle Processor: **A25597_UTM**
2. Start the run, remove the temporary paraffin plate seals (if present), then load the prepared processing plates in their positions when prompted by the instrument.
3. Load the sample plate (containing lysate, isopropanol, and Bead/RNase A Mix) at position 1 when prompted by the instrument.
4. When prompted by the instrument (approximately 30 minutes after initial start):
   a. Remove the Elution Plate from the instrument.
   b. Add 30 µL of DNA Elution Buffer 2 to each sample well.

      IMPORTANT! Add DNA Elution Buffer 2 immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

   c. Load the Elution Plate back onto the instrument, then press **Start**.
5. At the end of the run (approximately 5 minutes after the addition of DNA Elution Buffer 2), remove the Elution Plate from the instrument and seal immediately with a new clear adhesive film.
   - *(Optional)* Eluates can be transferred to a new storage plate after collection.
   - If you see excessive bead residue in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the DNA.

   IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:

- At 2–6°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

4 Prepare and run urinary tract microbiota profiling experiments with OpenArray™ plates

This chapter describes how to run profiling experiments with custom OpenArray™ plates using a QuantStudio™ 12K Flex Real–Time PCR System.

This chapter contains brief procedures. For detailed procedures, see the following documentation.

Document	Pub. No.
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
OpenArray™ Sample Tracker Software Quick Reference	4460657
OpenArray™ AccuFill™ System User Guide	4456986

Workflow: Microbiota profiling experiments with OpenArray™ plates

Genomic DNA
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Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software (page 25)

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Set up the PCR reactions in an OpenArray™ 384-well Sample Plate
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Set up the AccuFill™ instrument and software
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Transfer reactions to the OpenArray™ plate using the AccuFill™ instrument
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Seal the OpenArray™ plate
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Run the OpenArray™ plate on the QuantStudio™ 12K Flex Instrument
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Check the quality–control images
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Export and review urinary tract microbiota profiling data
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Required materials for OpenArray™ plate workflow

Unless otherwise indicated, all materials are available through **thermofisher.com**.

"MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Instruments, software, and equipment	
OpenArray™ Sample Tracker Software	—[1]
QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0	A24945
QuantStudio™ 12K Flex instrument with OpenArray™ block (AccuFill™ System)	4471090
Centrifuge, capable of spinning sample plates at 1,000 rpm	MLS
Plates and accessories	
OpenArray™ 384-well Sample Plates, black	4482221
Biomek™ Seal and Sample Foil Lids (for pre-plating step)	Beckman Coulter™ 538619
OpenArray™ AccuFill™ System Tips	4458107
QuantStudio™ 12K Flex OpenArray™ Accessories Kit[2]	4469576
Forceps	MLS
Reagents	
Genomic DNA	See page 12
(Optional) TaqMan™ Urinary Tract Microbiota Amplification Control	A39174
OpenArray™ plates with TaqMan™ Assays	• A39900 • Custom ordered[3]
TaqMan™ OpenArray™ Real-Time PCR Master Mix	4462164
Ethanol	MLS

[1] Included with the QuantStudio™ 12K Flex Software.

[2] Each kit contains the items needed to assemble up to 10 plates: 12 lids and plugs, 12 immersion fluid syringes, and 2 carriers. Each custom OpenArray™ plate order is shipped with accessories kits.

[3] See “Configure and order custom TaqMan™ OpenArray™ plates” on page 11.

Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software

Before generating 384-well sample plate layouts, see “One-time procedures” on page 32 to complete the following tasks:

- Set up optimized folder locations and software preferences.
- Download the TPF files for the OpenArray™ plates into the TPF Files folder.

1. Using a spreadsheet program, create a 96-well sample CSV file.
   a. Navigate to the following folder, then open the `96-Well Sample Plate 1.csv` template that is provided with the OpenArray™ Sample Tracker Software.

      <drive>`:\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples`

   b. **Save As** the template as a new 96-well sample CSV file. Save your 96-well sample CSV file in the **Sample Tracker 96-well Input** folder.

   c. Enter or copy the sample names into your 96-well sample CSV file.
2. Open the OpenArray™ Sample Tracker Software.
3. In the **Properties** screen, select **Gene Expression** for **Experiment Type**, then select the appropriate settings for **OpenArray™ Plate** and **Pipettor**.
4. In the **Samples** screen, click  **Import**, then select and import your 96-well sample CSV file that you created in step 1.
5. In the **Sample Mapping** screen, confirm that the samples for a single OpenArray™ plate are assigned to one color.

   Note: If necessary, correct the **OpenArray™ Plate** and **Pipettor** settings in the **Properties** screen.
6. In the **Sample Mapping** screen, click the **384-Well Plate** tab, then click **Export** ▸ **Export *.csv**.
7. Select **384-Well Plate (for AccuFill)**, enter a file name, then save the exported file.

Plate layouts for the 384-well sample plates are saved to individual CSV files in the **Sample Tracker 384-well CSV Files** folder.

Set up the PCR reactions in an OpenArray™ 384-well Sample Plate

IMPORTANT! The 4 × 12 area(s) of the OpenArray™ 384-well Sample Plate being filled must match the area(s) designated in the OpenArray™ Sample Tracker Software for that set of samples.

1. Remove an OpenArray™ plate from the freezer and set it aside. Allow it to come to room temperature in its unopened sleeve (~15 minutes).

   The OpenArray™ plate must be completely thawed before transferring reactions to it from the OpenArray™ 384-well Sample Plate created in this section.

2. Gently swirl the contents of the TaqMan™ OpenArray™ Real-Time PCR Master Mix to thoroughly mix. Do not invert the bottle.

3. Following the plate layout designated in the OpenArray™ Sample Tracker Software, add master mix, then DNA samples, to the wells of an OpenArray™ 384-well Sample Plate.

Component[1]	OpenArray™ Plate Format	
	18	56
	Volume per well	Volume per well
TaqMan™ OpenArray™ Real-Time PCR Master Mix	2.5 µL	2.5 µL
DNA sample	2.5 µL	2.5 µL
Total reaction volume	**5.0 µL**	**5.0 µL**

[1] *(Optional)* Include the TaqMan™ Urinary Tract Microbiota Amplification Control. For information about the amplification control, contact CustomControls@thermofisher.com.

4. Thoroughly mix each PCR reaction by pipetting up and down or by using the "mix" function on a multi-channel pipette.

5. Seal the OpenArray™ 384-well Sample Plate with an aluminum foil seal, remove the foil flap, then mark the edges of the filled 4 × 12 area with a pen.

6. Centrifuge the plate at 1,000 rpm for 1 minute.

7. Score the foil along the lines that were marked before centrifuging.

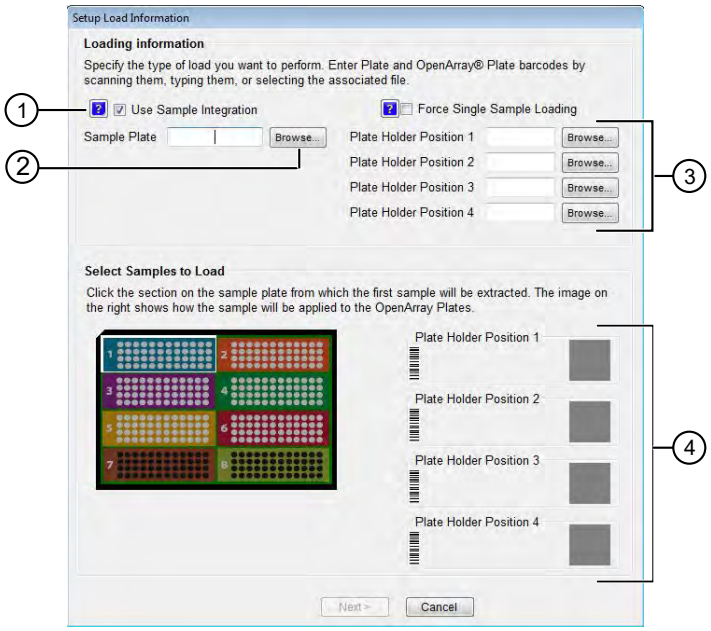
   Do not remove the foil from the scored area at this time.

If you make a sample layout error before the AccuFill™ procedure – Repeat “Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software” on page 25 with a corrected 96-well sample CSV file.

Set up the AccuFill™ instrument and software

IMPORTANT! Do not use OpenArray™ AccuFill™ System Tips that exceed the expiration date (shown on the outer box that contains the tip trays).

1. In the OpenArray™ AccuFill™ software, click **Setup and Load**.
   The **Setup Load Information** window appears.



① **Use Sample Integration** checkbox; select to integrate TPF files and the 384-well sample plate CSV file.
② **Browse** button; click to locate and select the 384-well sample plate CSV file. The button is displayed only if **Use Sample Integration** is selected.
③ **Browse** buttons; click to locate and select the TPF files for the OpenArray™ plates that will be placed in the corresponding **Plate Holder Position** on the deck of the AccuFill™ instrument. The buttons are displayed only if **Use Sample Integration** is selected.
④ **Plate Holder Position** corresponding to the position of the OpenArray™ plate on the deck of the AccuFill™ instrument.

2. Configure the **Loading Information** pane for sample integration using the 384-well sample plate CSV file and TPF files.
   a. In the **Loading Information** pane (top section of the window), ensure that the **Use Sample Integration** checkbox is selected.
   b. Click **Browse** to the right of the **Sample Plate** field, then select the 384-well sample plate CSV file that you generated with the OpenArray™ Sample Tracker Software in the Sample Tracker 384-well CSV Files folder.
   c. Click **Browse** to the right of the **Plate Holder Position** of the OpenArray™ plate, then select the TPF file for the OpenArray™ plate in the TPF Files folder.

3. In the **Select Samples to Load** pane (bottom section of the window), click the corresponding 4 × 12 area of the 384-well sample plate image, then click **Next**.
   The **Setup Deck** window is displayed.

4. In the AccuFill™ instrument, ensure that:
   - Tip boxes and tips are loaded as shown in the **Setup Deck** window.
   - The lids are removed from the tip boxes.
   - The waste bin in the instrument is emptied.

5. In the **Setup Deck** window, confirm that the deck is ready:
   - Select **The tips are configured as shown above**.
   - Select **The Waste Bin is empty**.

Transfer reactions to the OpenArray™ plate using the AccuFill™ instrument

IMPORTANT! Ensure that the OpenArray™ plate is thawed and that the entire plate is at room temperature.

1. Prepare the items needed to seal the loaded OpenArray™ plate (next section).

   Note: The OpenArray™ plate must be sealed promptly after being loaded with the reactions, as described here.

   - Ensure that the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0 is ready.
   - Gather and remove from their packaging the following: an OpenArray™ Lid, plug, syringe with OpenArray™ Immersion Fluid, and syringe tip.
   - Attach the syringe tip to the syringe, carefully push some of the fluid through the tip to remove air bubbles, then lay the syringe aside.

2. Load the OpenArray™ plate and the OpenArray™ 384-well Sample Plate into the AccuFill™ instrument.
   - **OpenArray™ plate**—Remove the plate from its sleeve, then place the plate in the appropriate plate holder position in the instrument.
     Ensure that the barcode on the OpenArray™ plate is facing left and the serial number is facing right.
   - **OpenArray™ 384-well Sample Plate**—Place the 384-well sample plate onto the deck of the instrument, then use forceps to peel the foil from the filled area of the plate.

3. Close the door of the AccuFill™ instrument.

4. In the AccuFill™ software **Setup Deck** window, select the following confirmations:
   - **The OpenArray Plate is in the Plate Holder**
   - **Remove foil from the highlighted section of the Sample Plate**

5. Click **Load**.

6. As soon as the **Remove OpenArray Plate** window appears, open the instrument door and remove the loaded OpenArray™ plate.

7. Proceed immediately to seal the OpenArray™ plate (next section).

Note: For best results, seal the OpenArray™ plate within 90 seconds of completion of loading to prevent evaporation.

Seal the OpenArray™ plate

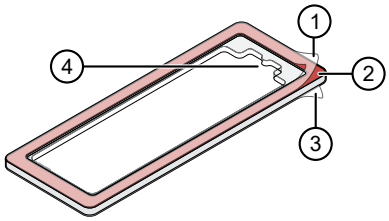
IMPORTANT! Throughout this procedure, handle the OpenArray™ plate and the OpenArray™ Case only by the edges.

Note: The OpenArray™ Case consists of the sealed OpenArray™ plate and the OpenArray™ Lid.

1. Place the newly loaded OpenArray™ plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

   Ensure that the barcode is facing left and the serial number is facing right.

2. From the OpenArray™ Lid, remove the clear protective film from the *inside* of the lid and the red adhesive-protective strip from around the edge of the lid.



OpenArray™ Lid

① Protective film on inside of the lid (remove before *sealing*)
② Red adhesive-protective strip (remove before *sealing*)
③ Protective film on the outside of the lid (remove before *running*)
④ Notched end (align with serial number on plate)

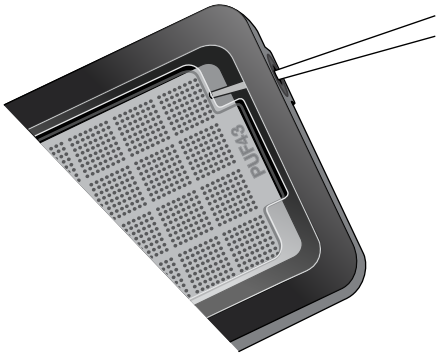
3. Seat the lid on the OpenArray™ plate with the lid adhesive against the plate.

4. Engage the press mechanism until the green flashing light changes to a steady green light (approximately 20 seconds).

5. Disengage the press and remove the OpenArray™ Case.

6. While holding the case by its edges, insert the prepared syringe tip into the port in the case, then carefully inject OpenArray™ Immersion Fluid until the case is filled.

   Note:
   - Minimize creation of air bubbles when you dispense the fluid.
   - Leave a small bubble at the fill point to prevent fluid leaks during the instrument run.



The syringe tip must be in front of the array when filling the case with immersion fluid.

7. While holding the case *vertically*, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port of the case, then rotate clockwise until the black handle breaks off.

   IMPORTANT! To avoid leaking of immersion fluid, hold the case *vertically* and rotate the plug slowly.

   If the plug handle breaks off prematurely, use a Phillips #0 screwdriver to complete this step.

8. If needed, clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

Run the OpenArray™ plate on the QuantStudio™ 12K Flex Instrument

You can run up to four OpenArray™ plates at one time on the QuantStudio™ 12K Flex Instrument.

1. On the QuantStudio™ 12K Flex Instrument touchscreen, touch  to extend the instrument tray arm.

2. Remove the clear protective film from the outside of the OpenArray™ case (sealed plate + lid).

3. Place the OpenArray™ case on the tray arm plate adapter.
   - Support the case from underneath the tray arm to prevent the case from slipping through the adapter.
   - Ensure that the plate barcode and serial number are facing the front of the instrument.

4. Touch  to retract the instrument tray arm.

5. In the **Home** screen of the QuantStudio™ 12K Flex Software, in the **Run** pane, click **OpenArray**.
6. In the **Select Instrument** pane, select your instrument.
7. Click **Get Plate IDs** to import the barcode of the OpenArray™ plate.
   Once the OpenArray™ serial number appears, the loaded TPF file corresponding to the plate should appear in the **Setup File** field.
   If the TPF file does not appear, click **Browse**, then select the correct loaded TPF file from the **Loaded TPF** folder.
8. *(Optional)* Click **Browse** to change the **Experiment File Location**.
9. *(Optional)* Change the software-determined **Experiment File Name**.
10. Click **Start Run**.

    Note: The instrument pauses at 41 or 42 seconds prior to the end of the run. Wait for the system to complete the run before opening the EDS file.

11. Transfer the EDS file from the instrument to an accessible location for analysis.
12. Check the QC images for loading issues or leaks.

Check the quality–control images

Check the quality–control (QC) images before analysis. Images can be viewed using ImageJ, an open–source software available from the NIH at **imagej.nih.gov/ig**. For additional information, see Appendix A, “Troubleshooting”.

1. In the QuantStudio™ 12K Flex Software **Export** screen, click **Browse**, then create a uniquely–named folder for the QC images export.

   IMPORTANT! Create a new folder for images each time. Exporting a second run to the same folder overwrites the images.

2. Click **Export QC Images** at the bottom of the screen.
3. View the following ROX™ image to check for loading quality issues:
   - POST-READ_CHANNEL_4.tiff
4. Check the following spotfinding images for leaks or other displaced sample issues.
   - s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff
   - s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff

   Note: The “cp#” in the image file name refers to array positions 1 through 4 within the instrument.

5. If a problem is found, view the following pre-run spotfinding image to determine whether the issue existed before cycling:
   - s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff
6. View the following FAM™ images to check for fluorescent abnormalities and to confirm any problem seen in the spotfinding images:
   - STAGE2_CYCLE1_CHANNEL_1.tiff
   - STAGE2_CYCLE40_CHANNEL_1.tiff
7. Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

One-time procedures

Set up default folders and software preferences

This procedure simplifies the file locations used in the AccuFill™ software.

Set up the default file locations and preferences before using the OpenArray™ AccuFill™ system for the first time. You must be logged in as an administrator.

1. Create the following four folders in a convenient location on the same computer drive as the AccuFill™ software:
   - **TPF Files**
   - **Sample Tracker 96-well Input**
   - **Sample Tracker 384-well CSV Files**
   - **Loaded TPF Files**
2. *(Optional)* Copy a template file into the OpenArray™ Sample Tracker Software folder.
   - Navigate to this folder on your computer: `<drive>:\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples`.
   - Copy the `96-Well Sample Plate 1.csv` template file, which is provided with the OpenArray™ Sample Tracker Software.
   - Paste the template file into the **Sample Tracker 96-well Input** folder.

3. In the OpenArray™ Sample Tracker Software, select **View ▸ Preferences**, then enter the following preferences:

Field	Selection
Experiment Type	**Gene Expression**
OpenArray™ Plate	Select the OpenArray™ format that will be run most often, such as **Gene Expression – 56.**
Pipettor	**Fixed** or **Adjustable** tip spacing
Import Data Directory	**Sample Tracker 96-well Input**
Export Data Directory	**Sample Tracker 384-well CSV Files**

4. In the AccuFill™ software, select **Instrument ▸ Edit Preferences**, then:
   a. Select **Require Sample Integration**.
   b. Select the folders indicated in this table:

AccuFill™ folder	Default folder	Folder contents
OpenArray™ Plate File Input Folder	**TPF Files**	TPF files for the OpenArray™ plates, with assay name and location
Sample Plate File Folder	**Sample Tracker 384-well CSV Files**	CSV 384-well sample plate layout files
Loaded OpenArray™ Plate File Folder	**Loaded TPF Files**	Integrated TPF files generated during processing with the AccuFill™ software.

5. In the QuantStudio™ 12K Flex Software, select **Tools ▸ Preferences ▸ OpenArray**, then select the **Loaded TPF Files** folder for the software **Setup Folder**.

Note: If the QuantStudio™ 12K Flex Software is not on the same computer as the AccuFill™ software, transfer the loaded TPF files to the computer running the QuantStudio™ 12K Flex Software.

Download TPF files

Set up the optimized folder locations and software preferences before downloading TPF files. See “Set up default folders and software preferences” on page 32.

To download TPF files for custom OpenArray™ plates, you need the **Lot#** and the **Serial#** from the packaging of each OpenArray™ plate.

1. Go to **thermofisher.com/OA-platefiles**.
2. From the **Select Your Product** dropdown list, select **TaqMan™ OpenArray™ Custom Gene Expression/Genotyping Plates**.
3. Select the desired option for downloading either only the TPF files or both the TPF files and the AIF files.
4. Enter the **Lot#** and the **Serial#**, then click **Submit**.

   Note: The **Serial#** is case-sensitive.

5. Save the TPF files to the desktop **TPF Files** folder.

   Note: Do not create sub-folders in the **TPF Files** folder. The software cannot access sub-folders.

5 Export and review urinary tract microbiota profiling data

Export data

1. Open an EDS file in the QuantStudio™ 12K Flex Software.
2. In the **Experiment Menu** pane, click **Export**.
3. Click **Load Export Set** (bottom of the screen), select **GE_export_setting**, then click **OK**.
4. Select **.xlsx** from the **File Type** dropdown list (top-right of the screen).
5. *(Optional)* Perform any of the following actions to customize the file export.
   - Click **Browse** to select a new **Export File Location**.
   - Enter a new file name in the **Export File Name** text field.
   - Click the **Results** tab, then select the content to export.
6. Click **Start Export** (bottom of the screen).
   If **Open file(s) when export is complete** is selected, then the file automatically opens. If the option is not selected, navigate to and open the exported XLSX file.

Prepare exported data for analysis

1. Open the exported XLSX data file.
2. Ensure that the barcode, run conditions, and all selected data columns were exported correctly.
3. Scroll down to the data rows, select the headers and data, then copy-paste into a new worksheet.
4. Rename the new worksheet **Data Table_*Run File Name***.
5. *(Optional)* To combine data from multiple OpenArray™ plates:
   a. Insert a **Barcode** column in the **Data Table** worksheet to track OpenArray™ barcodes.

b. Copy-paste the barcode numbers to the appropriate cells in the new **Barcode** column.

6. Find-replace all "**Undetermined**" values with an empty cell (no value) in the **C_{rt}** column. This step ensures an exact count of C_{rt} values.

7. Delete rows that do not contain run data.

Review results

Note: These guidelines apply to results from experiments that included three or more technical replicates.

Note: We encourage testing and establishing your own C_{rt} cut-off value for each assay to achieve high sensitivity and specificity.

1. Review the exported data for through-hole results that may require special attention.

2. Consider filtering out from analysis through-holes with the following values:

Parameter to examine	Consider filtering out through-holes if…
C_{rt}	**C_{rt}** ≥ 31
C_q Confidence	**C_q Conf** < 0.8 • RNase P (Hs04930436_g1) — acceptable range is 0.7 – 1.0
Amp Score	Amp Score < 1.2

Note: Through-holes with unexpected C_{rt} values can also be identified by reviewing the Amplification Plot (see page 38).

3. Review through-holes with $C_{rt} > 28$ and ensure that the C_{rt} values are reproducible in all technical replicates.

Note: $C_{rt} = 28$ is approximately equal to 1 copy of the target sequence in a reaction.

4. Take note of technical replicates with mean $C_{rt} \leq 25$ and a high standard deviation (> 0.5). The data from these through-holes might require further review.

5. Ensure that at least half of the replicates amplified adequately and pass your review specifications.

6. Use your preferred method to analyze the data.

Fields for reviewing results with pivot tables

To review results using the pivot table feature of a spreadsheet program, you can use the following settings.

Note: For the "Average of" and "StdDev of" summarizations, use the appropriate source field (**C_{rt}**, **Amp Score**, or **C_q Conf**), then choose the calculation type.

Area of pivot table	Fields to add	
	Target-oriented view	Sample-oriented view
Filters	—	Sample Name[1]
Columns	Sample Name	—
Rows	Target Name	Target Name
Values	Average of C_{rt}	Average of C_{rt}
	StdDev of C_{rt}[2]	StdDev of C_{rt}[2]
	Count of C_{rt}	Count of C_{rt}
	—	Average of Amp Score
	—	Average of C_q Conf

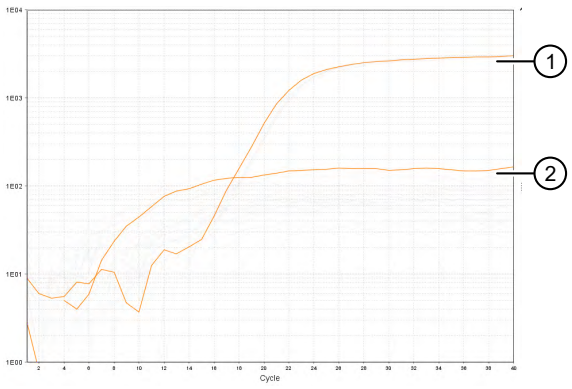
[1] To see individual sample results, select the sample from the dropdown list next to the **Sample Name** header.

[2] A **Values** field will automatically appear in the **Column Labels** area.

A Troubleshooting

Troubleshoot DNA isolation errors

Troubleshoot unexpected C_{rt} values

Observation	Possible cause	Recommended action
Unexpected C_{rt} values in the amplification plot  ① Expected C_{rt} value (noted in most replicates) ② Unexpected C_{rt} value (too low)	Unexpectedly low C_{rt} values (< 10) — Signal variation or noise in early PCR cycles	Review amplification curves, AmpScore, and C_q Confidence values.
		Consider filtering C_{rt} values from analysis.
		Compare replicates, if available.
		Repeat the experiment.
	Unexpectedly high C_{rt} values (< 28) — Sporadic amplification	Review amplification curves, AmpScore, and C_q Confidence values.
		Consider filtering C_{rt} values from analysis if < 50% of replicates amplified at similar levels.
		Repeat the experiment.

Troubleshoot with cycling and imaging run images (QC images)

Many problems with OpenArray™ results can be diagnosed by examining the quality control (QC) images taken at various points during a cycling/imaging run.

The QC images are fluorescent or reflected light images taken before, during, and after cycling. They may require adjustment to make image features visible. To view the images, we recommend that you install the free software program ImageJ, which allows you to easily manipulate the images in ways that other image viewers cannot.

1. In the QuantStudio™ 12K Flex Software **Export** screen  :
   a. Click **Browse** to select a uniquely-named folder for the QC images export.
   b. Click **Export QC Images** (bottom of screen).

IMPORTANT! Select a new folder for images each time; exporting a second run to the same folder overwrites the images.

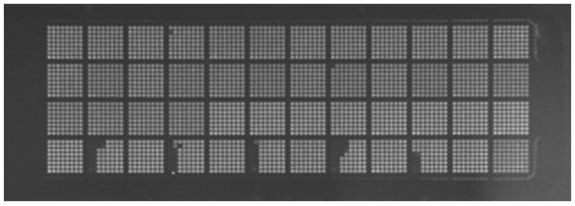
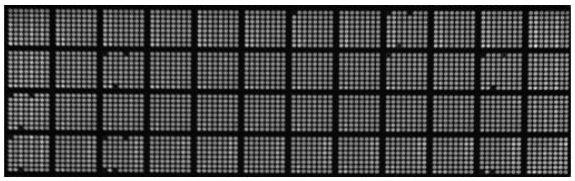
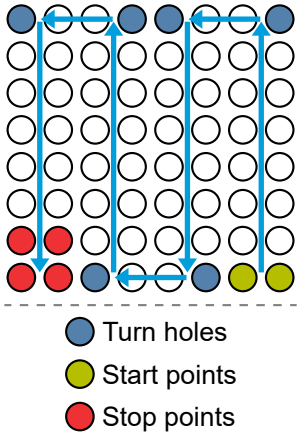
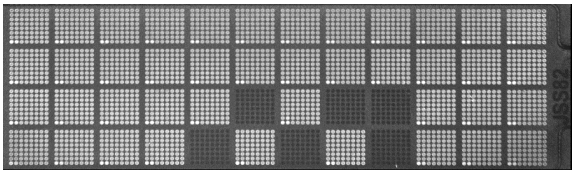
2. Use ImageJ to view the images of interest.

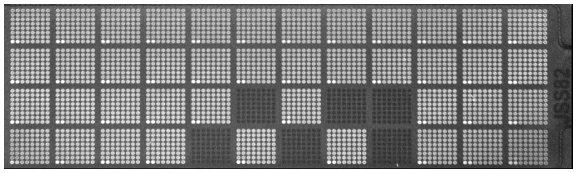
To...	View image...	Image description
Confirm the identity of images within a folder	BARCODE IMAGE.tiff	Reflected light image of the entire OpenArray™ plate
Evaluate the loading quality	PRE-READ_CHANNEL_4.tiff POST-READ_CHANNEL_4.tiff	Pre- and post-ROX™ dye images
Check for existing contamination on the case and/or heated cover	s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff[1]	Pre-run reflected light spotfinding image (used by the software to determine the location of the holes)
Identify potential leaks or other contamination	s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff[1]	Mid-run reflected light spotfinding image
	s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff[1]	Post-run reflected light spotfinding image
Look at patterns in the fluorescent data (for example, gradients)	STAGEx_CYCLEy_CHANNEL_1.tiff	FAM™ images at a particular cycle (y) of a particular stage (x) of the run.

[1] The "cp#" in the image file name refers to the array position (1–4) within the QuantStudio™ 12K Flex Real-Time PCR Instrument.

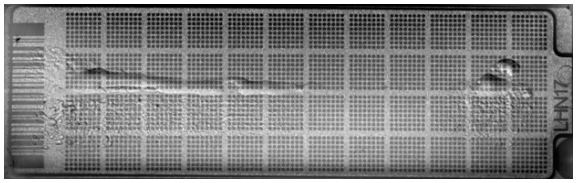
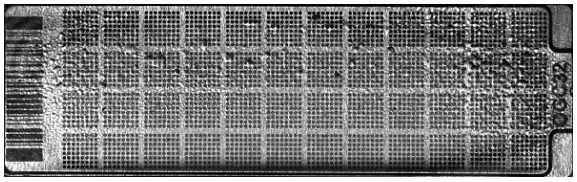
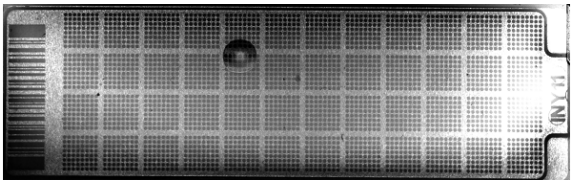
3. *(Optional)* Adjust the images for brightness and/or contrast to make image features visible.
   a. Open the image in ImageJ.
   b. Select **Image** ▸ **Adjust Brightness/Contrast** (or press **Ctrl+Shift+C**).
   c. Click **Auto** or adjust the sliders until the features of interest in the image are visible.

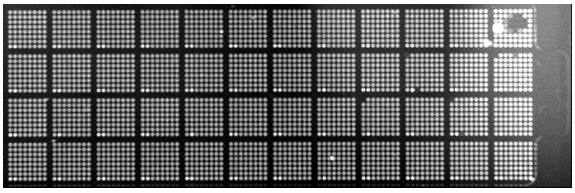
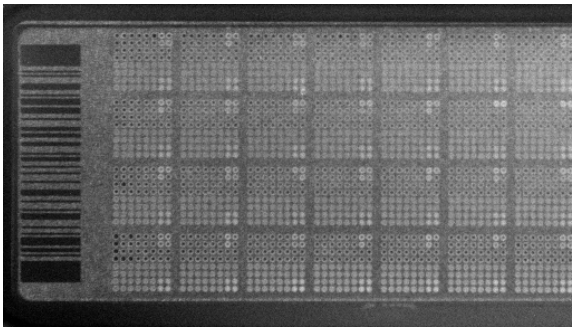
AccuFill™ instrument plate loading errors

Observation	Possible cause	Recommended action
There are empty through-holes 	Insufficient sample was added to the 384-well Sample Plate.	Use proper pipetting techniques. Ensure that there are no air bubbles in the pipette tips after sample aspiration.
	Reaction mix (sample + master mix) is not at the bottom of the 384-well Sample Plate.	Centrifuge the plate at 1,000 rpm for 60 seconds.
Turn-holes are repeatedly missed 	AccuFill™ instrument is aligned too far to the left or to the right. Systematic loading problems can occur with the AccuFill™ instrument, which indicates a need for service. For example, when turn-holes are repeatedly missed across multiple subarrays, service is required. Turn-holes are where the AccuFill™ instrument changes direction during sample loading. 	Contact your local field service engineer.
Entire subarrays are missing 	The sample/master mix was not added to particular wells in the 384-well Sample Plate.	Visually inspect the plate to ensure that the wells have sample/master mix.
	Stuck tip mandrel on AccuFill™ instrument may need cleaning.	Contact your local field service engineer.

Observation	Possible cause	Recommended action
Entire subarrays are missing  *(continued)*	Pipette tip was not loaded on mandrel.	Contact your local field service engineer for frequent occurrences (infrequent occurrences can be due to a poorly molded tip).

OpenArray™ plate assembly and handling errors

Observation	Possible cause	Recommended action
Case leaks and bubbles inside the case    Improper sealing of the OpenArray™ plate in the OpenArray™ Case can lead to immersion fluid leaks or bubble formation inside the case, leading to uneven heating and imaging throughout PCR and to poor quality data. The images above are examples of OpenArray™ plates that have been affected by immersion fluid leaks. The images show where leaked fluid has condensed on the underside of the heated cover	Plate press was not engaged for at least 20 seconds.	Fully engage the plate press for at least 20 seconds.
	Damaged lid adhesive.	Remove the liner and visually inspect the lid adhesives for defects. Ensure that adhesive is not damaged or warped.
	Damaged fill port screw gasket.	Visually inspect the screw to ensure that the orange gasket is present and not damaged.
	Damaged fill port screw assembly. Breaks off too easily.	The screw may be mis-threaded: unscrew it and use a new screw assembly.
	Oily lid or case from immersion fluid overflow.	Wipe off excess overflow of immersion fluid from the lid, case bottom, and crevices with 70% isopropyl alcohol, using a lint-free cloth (the cloth included with the OpenArray™ plate is acceptable).
	Immersion fluid was exposed to air for too long.	Do not remove the immersion fluid syringe cap or draw air bubbles into the syringe until you are ready to load.

Observation	Possible cause	Recommended action
windows and obscured the view of the through-holes. The best image in which to detect leaks is the s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff image. This image is taken at the start of cycling, which is where most leaks occur. See "Troubleshoot with cycling and imaging run images (QC images)" on page 39.	Too large of a bubble inside the OpenArray™ Case after sealing.	Minimize the size of the bubble by tilting the OpenArray™ Case so that the fill port is at the highest point. Slowly fill the case with immersion fluid until only a small air bubble remains. Attach the screw and wipe off any excess oil that may have spilled onto the case.
	Damaged plate press, leading to uneven pressure.	Contact your field service engineer if you suspect that your plate press may be damaged.
Sample blow-out during the addition of immersion fluid 	The reactions in A12 were compromised during the addition of immersion fluid. Injecting the immersion fluid too quickly can purge the sample out of the through-holes near the fill port. Often this is caused by the user not purging the syringe slightly before use.	Dispense a small amount of immersion fluid onto a paper towel before use to ensure smooth operation of the syringe.
Evaporation of reaction mixture in through-holes 	Too much time elapsed before the plate was sealed with lid and immersion fluid. In this example, the top half of each subarray was intentionally left open to the environment to demonstrate the effect of evaporation. "Donuts" are a result of the evaporated fluid in the though-holes.	Add immersion fluid as soon as the case is removed from the plate press to minimize the likelihood of evaporation, then seal the case with the lid.

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication Number
Urinary Tract Microbiota Profiling Experiments Application Guide	MAN0017750
DNA isolation for Urinary Tract Microbiota Profiling Experiments Quick Reference	MAN0017751
OpenArray™ Urinary Tract Microbiota Profiling Experiments Quick Reference	MAN0017752
TaqMan™ Urinary Tract Microbiota Amplification Control Product Information Sheet	MAN0017753
TaqMan™ Universal DNA Spike In Control Product Information Sheet	MAN0017852
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
OpenArray™ Sample Tracker Software Quick Reference	4460657
OpenArray™ AccuFill™ System User Guide	4456986

Symbols that may be displayed on the instrument, in the software, or in this guide

Symbol	Description	Symbol	Description
	MANUFACTURER		DATE OF MANUFACTURE
	CATALOG NUMBER		SERIAL NUMBER
	CONSULT INSTRUCTIONS FOR USE[1]		CAUTION[1]

[1] "Documentation and support" on page 46

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  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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